CLAIMS

What is claimed and desired to be secured by Letters Patent is as follows:

1. The recombinant mycobacterium strain GPM259 on deposit with the American Type Culture Collection as Accession No. _____.
2. The recombinant mycobacterium strain GPM260 on deposit with the American Type Culture Collection as Accession No. _____.
3. The recombinant mycobacterium strain GPM265 on deposit with the American Type Culture Collection as Accession No. _____.
4. The recombinant plasmid pBUN250, said plasmid contained within the transformed mycobacterium strain of claim 1.
5. The recombinant plasmid pBUN250, said plasmid contained within the transformed mycobacterium strain of claim 2.
6. The recombinant plasmid pBUN276, said plasmid contained within the transformed mycobacterium strain of claim 3.
7. A transformed microorganism comprising a host mycobacterium and the plasmid of claim 4.
8. The transformed microorganism of claim 6, wherein the host microorganism comprises a mycobacterium strain selected from the group consisting of *M. smegmatis, M. tuberculosis, M. bovis, M. africanum, M. microti, M. leprae, M. avium, M. intracellular, M. paratuberculosis, M. ulcerans, M. marinum*, and any genetic variants thereof.
9. A transformed microorganism comprising a host mycobacterium and the plasmid of claim 5.
10. The transformed microorganism of claim 9, wherein the host microorganism comprises a mycobacterium strain selected from the group consisting of *M. smegmatis, M. tuberculosis, M. bovis, M. africanum, M. microti, M. leprae, M.avium, M. intracellular, M. paratuberculosis, M. ulcerans, M. marinum*, and any genetic variants thereof.

11. A transformed microorganism comprising a host mycobacterium and the plasmid of claim 6.

12. The transformed microorganism of claim 11, wherein the host microorganism comprises a mycobacterium strain selected from the group consisting of *M. smegmatis, M. tuberculosis, M. bovis, M. africanum, M. microti, M. leprae, M. avium, M. intracellular, M. paratuberculosis, M. ulcerans, M. marinum*, and any genetic variants thereof.

13. An isolated polypeptide, comprising a mycobacterial D-alanine ligase enzyme expressed by the recombinant mycobacterium strain of claim 1.

14. An isolated polypeptide, comprising a mycobacterial D-alanine ligase enzyme expressed by the recombinant mycobacterium strain of claim 2.

15. An isolated polypeptide, comprising a mycobacterial D-alanine ligase enzyme expressed by the recombinant mycobacterium strain of claim 3.

16. A method for screening for the presence or absence of D-alanine ligase inhibition by a test sample, comprising:

    (a) incubating a culture of recombinant mycobacterium strain, wherein the strain is selected from the group consisting of a recombinant mycobacterium strain of claim 1, a recombinant mycobacterium strain of claim 2, and a recombinant mycobacterium strain of claim 3, in the presence of the test sample; and

    (b) assessing the effect the test sample has on the growth of said recombinant mycobacterium strain.

17. A method for screening for drugs which target the D-alanine branch of mycobacterial peptidoglycan synthesis, wherein said drugs are D-cycloserine or compounds structurally related to D-cycloserine, comprising:

(a) incubating a culture of a recombinant mycobacterium strain selected from the group consisting of a recombinant mycobacterium strain of claim 1, a recombinant mycobacterium strain of claim 2, and a recombinant mycobacterium strain of claim 3, in the presence of a drug; and

(b) assessing the effect the drug has on the growth of said recombinant mycobacterium strain.

18. A method for assaying for enzyme activity of D-alanine ligase enzyme isolated from a recombinant mycobacterium strain selected from the group consisting of a culture of recombinant mycobacterium strain of claim 1, recombinant mycobacterium strain of claim 2, and recombinant mycobacterium strain of claim 3.

19. The method of claim 18, wherein said assay is a thin-layer chromatography based method, and wherein activity is assayed by measuring the production of D-alanine:D-alanine dipeptide and D-alanine.

20. The method of claim 18 wherein said assay is a phosphate release assay.

21. A method for screening for drugs which target the D-alanine branch of mycobacterial peptidoglycan synthesis, wherein said drugs are structurally related to D-cycloserine, comprising the method of claim 18.

22. A method for analyzing the mechanism of action of antimycobacterial drugs on production of D-alanine by mycobacterial strains, comprising analysis of free amino acid pool, whereby said analysis indicates which pathways are affected by an antimycobacterial drug.

23. A vaccine composition comprising a live-attenuated recombinant mycobacterium strain, wherein said live-attenuated recombinant mycobacterium strain comprises: an episomal

copy of a *ddl* gene under the control of a promoter which is not expressed *in vivo*; and an inactivated native *ddl* gene.